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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Frank LUYTEN et al.	Confirmation No.:	5817
Serial No.:	10/089,994	Art Unit:	1632
Filed:	July 2, 2002	Examiner:	Thaia N. Ton
Customer No.:	21559		
Title:	ISOLATION OF PRECURSOR CELLS AND THEIR USE FOR TISSUE REPAIR		

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. FRANK LUYTEN

1. I am a named inventor on the above-referenced patent application.
2. I am a Professor at the University of Leuven. I have over 20 years of experience in the field of Rheumatology. A copy of my curriculum vitae is attached.
3. I have read and understand the Final Office Action mailed December 15, 2006. In particular, I understand that the Examiner has questioned whether the evaluation of cartilage production in nude mice is a reliable model for a therapeutic effect. The Examiner has indicated that the working examples show *in vivo* implantation of the cells by intramuscular injection of the cells into nude mice, which, the Examiner has indicated is not considered analogous to what could be considered a therapeutic treatment. The Examiner has indicated that the examples fail "to correlate to a therapeutic result in utilizing the claimed cells" (see, Final Office Action, page 8, second paragraph).
4. In the invention described in the application under consideration, it is demonstrated that, based on the correlation with cartilage production upon injection into nude mice, markers can be identified, which are representative of the ability of the cells to produce stable hyaline cartilage when injected *in vivo*. It was found that the ability of cells to produce stable hyaline cartilage when injected *in*

vivo, is linked to the expression of specific markers by these cells prior to injection. Moreover it was found that precursor cells of chondrocytes are similarly capable of producing stable hyaline cartilage when injected *in vivo*, and that accordingly, representative markers of this cartilage-forming ability can be identified.

5. That a cell population expressing the markers for cartilage-forming ability are indeed also capable of producing stable hyaline cartilage *in vivo*, when injected into a cartilage defect is further supported by the enclosed data (Annex). These data demonstrate that the expression, by a cell population obtained from a biopsy, of markers which have been identified to be representative of cartilage forming ability *in vivo* using the nude mouse model, is indicative of the ability of the cell population of producing stable hyaline cartilage when injected into a cartilage defect, and thus representative of the therapeutic potential of that cell population. Accordingly, these data confirm that the markers identified by the *in vivo* nude mouse model, are reliable tools to identify whether or not a cell population obtained from a biopsy is suitable for use in the therapy of cartilage defects using autologous cell transplantation. The application demonstrates that, using the same *in vivo* nude mouse model, the relevant markers for chondrocyte precursors cells were identified. As demonstrated in the enclosed data, the markers allow predictable determination of whether or not these cells can improve cartilage formation in cartilage defects. Accordingly it is submitted we have thus provided methods and tools for identifying therapeutically useful precursor cell populations.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date:

June 19, 2007

Dr. Frank Luyten

ANNEX

Experimental data**1) Identification of markers for the ability to produce stable hyaline cartilage in vivo using the nude mouse model**

Markers for the ability of cells to produce stable hyaline cartilage *in vivo* were identified using the nude mice model as described in the specification. Briefly, cell populations were injected intramuscularly into nude mice and the ability of the cell populations to produce stable hyaline cartilage was monitored. The markers specifically expressed by those populations which, when injected, led to stable hyaline cartilage formation in mice, were identified as markers of chondrocyte phenotypic stability.

2) Use of the markers to identify populations for implantation into a cartilage defect

In a randomized, well-controlled, level I-1a evidence clinical trial, the biomarkers identified during the phase (1) were used to identify by molecular screening *in vitro* cartilage biopsies from patient suffering from osteoarthritis, populations of cells capable of producing hyaline cartilage *in vivo* for re-injection into the patients. Patients were first evaluated to obtain a Baseline score (BSL) of the defect. Healthy cartilage was then harvested from an unaffected part of the joint. The cell populations obtained from the were assessed for the expression of the relevant markers by molecular screening. The expression of these markers was attributed a score (C-C). The cells were then further cultured before being re-implanted into the cartilage defect in the patient.

Clinical improvement was evaluated at 12 to 18 months after implantation.

3) Correlation between the expression of the biomarkers and the clinical outcome

The score used to measure clinical improvement is the well-validated Knee Osteoarthritis Outcome Score (KOOS). Another measurement was done based on patient-reported questionnaire that measures pain, symptoms, activity of daily living, sports and recreational activities and quality of Life (QOL score).

A statistically significant positive correlation was observed between the expression of the biomarkers for chondrocyte phenotypic stability by the cell populations prior to injection, and the average clinical improvement as measured by KOOS and QOL scores (see, Figures 1 and 2, respectively).

4) Conclusion

Based on the above it is concluded that the expression by a cell population of the markers identified in the nude mouse model as indicative of chondrocyte phenotypic stability, is representative of the therapeutic potential of the cells when injected in a cartilage defect.

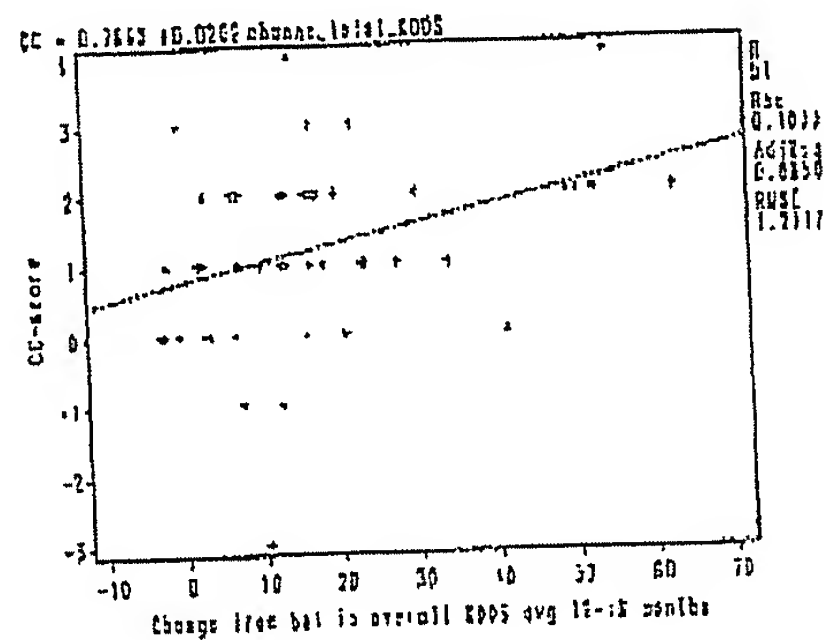


Figure 1: correlation between CC-score and change in overall KOOS (Pearson correlation coefficient: 0.32138; $p=0.0215$)

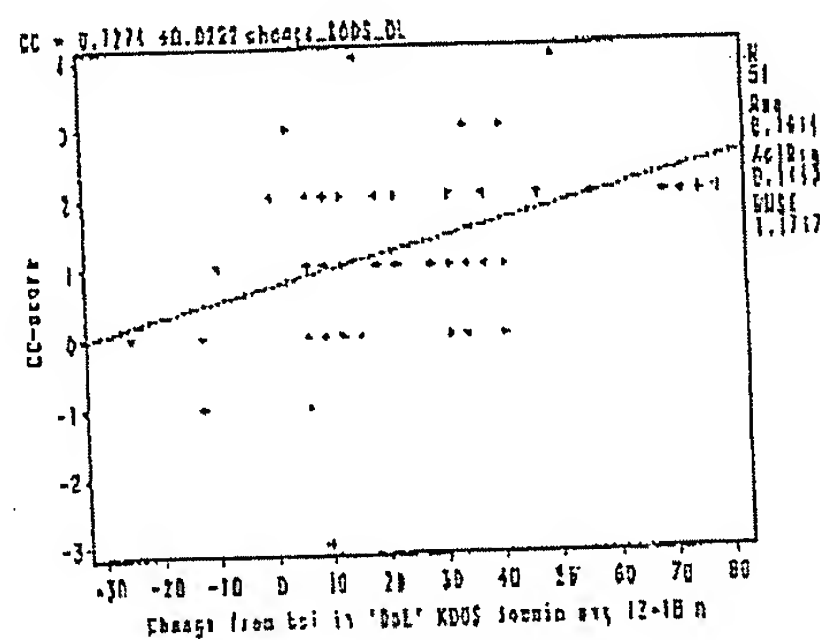


Figure 2: correlation between CC-score and change in the quality of life subscore of KOOS (Pearson correlation coefficient: 0.40172; $p=0.0035$)

CURRICULUM VITAE

NAME: FRANK PROSPER JOZEF LUYTEN

PLACE OF BIRTH: Oostende, Belgium

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CITIZENSHIP: Belgium

LANGUAGES: Dutch, French, English, German

MARITAL STATUS: Married to Catheline de Jonge, 3 children

POSITIONS:

- 1997-present Professor, Faculty of Medicine, KU Leuven and Chairman of the Division of Rheumatology and of the Department of Musculoskeletal Sciences, University Hospitals, Herestraat 49, B-3000 Leuven, Belgium
- 1993-1997 Senior Scientist
Chief, Developmental Biology Unit
Craniofacial and Skeletal Diseases Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD, USA
- 1988-1992 Visiting Associate, Bone Cell Biology Section, Laboratory of Cellular Development and Oncology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD, USA
- 1986-1988 International Fogarty Fellow, Bone Cell Biology Section, Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD, USA
- 1983-1986 Resident and Staff Member, Department of Rheumatology, University Hospital Ghent, Belgium, Europe
- 1980-1983 Resident, Department of Internal Medicine, University Hospital Ghent, Belgium, Europe

GCP/OHC statement:

By signing this CV, I state that I am fully aware of GCP and ICH guidelines for clinical trials, and that I am trained with regarding to these guidelines during several investigator meetings and initiation visits. Last training: June 2004.

EDUCATION and DEGREES:

- Bachelor of Medicine: July, 1976, cum laude.
University of Ghent, Belgium
- Medical Doctor (M.D.): July, 1980, magna cum laude.
University of Ghent, Belgium
- Doctor in Bio-Medical Sciences (Ph.D.):
June, 1986, maxima cum laude.
University of Ghent, Belgium
- Board Certified Rheumatologist (Belgium, W-Europe)
July, 1986

SCIENTIFIC HONORS/AWARDS/FELLOWSHIPS:

- Ciba Award for Research in Rheumatology, 1984.
for the work entitled "Chondrocytes in situ: a long-term organ culture model to study the repair of human articular cartilage", F.P.Luyten
- NATO Research Fellowship, 1986-1987.
- International Fogarty Research Fellowship, 1986-1987.
- NIH Fogarty Fellowships as Visiting Associate and Visiting Scientist, 1988-1997.
- Expert Member of the Scientific Advisory Board, Kennedy Institute for Rheumatology, UK, 1996.
- Member of the Study Section Oral Biology/Medicine, NIH, USA, 1996-1997.
- Expert reviewer for the Human Science Frontier Program, 1996-1997.
- Expert-Reviewer INSERM 2002-
- Scientific Advisor- Instituts de Biotherapie-, Montpellier, France

PATENT APPLICATIONS:

PCT/US94/12814

Cartilage-derived Morphogenetic Proteins, novel members of the TGF- β superfamily"

Principal inventor: F. P. Luyten

US Application 10/379,830

PCT/US97/18362 – 10/014.055

Isolation and use of tissue growth inducing FRZB protein

Principal inventor: F. P. Luyten

Publication N° US-2003-0139591

US patent 09/851,921 – US Patent N° 6,617,161

Serum-free cell growth medium

Principal Inventor: F. P. Luyten

WO2004012503

Compositions comprising muscle progenitor cells and uses thereof.

Inventor(s): DE BARI, Cosimo; LUYTEN, Frank; DELL'ACCIO, Francesco

Filed 30/07/2003

Published 12/02/2004

Applicant Tigenix

WO2003000724

Polynucleotide sequences and vectors useful for the prevention or treatment of bone- or cartilage-related disorders

Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco

Filed 08/03/2002

Published 03/01/2003

Applicant Tigenix

GB2385052

Treatment of spondyloarthropathies

Inventor(s): Luyten, Frank; Lories, Rik

Filed 20020205

Published 20030813

Applicant K U Leuven Research & Development

WO2003066081

BMP inhibitors for the treatment of spondyloarthropathies

Inventor(s): LUYTEN, Frank; LORIES, Rik

Filed 05/02/2003

Published 14/08/2003

Applicant K U Leuven Research & Development

US20030235813

In vivo assay and molecular markers for testing the phenotypic stability of cell populations, and selecting cell populations for autologous transplantation

Inventor(s): Luyten, Frank; De Bari, Cosimo; Dell'Accio, Francesco

Filed 24/04/2003

Published 25/12/2003

Applicant Tigenix

WO0124833

In vivo assay for testing the phenotypic stability

Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco

Filed 06/10/2000

Published 12/04/2001

Applicant Tigenix

EP1218037

In vivo assay for testing the phenotypic stability

Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco

Filed 06/10/2000

Published 03/07/2002

Applicant Tigenix

WO0125402

Isolation of precursor cells and their use for tissue repair

Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco

Filed 06/10/2000

Published 12/04/2001

Applicant Tigenix

EP1282690

Isolation of precursor cells and their use for tissue repair

Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco

Filed 06/10/2000

Published 12/02/2003

Applicant Tigenix

US20030176683 A1

Cartilage-derived morphogenetic proteins

Inventor(s): Luyten, Frank, P.; Moos, Malcolm; Chang, Steven, Chao-Huan

Filed 03/03/2003

Published 18/09/2003

Applicant

US20010037017 A1

DNA molecules encoding cartilage-derived morphogenetic proteins

Inventor(s): Luyten, Frank, P.; Moos, Malcolm; Chang, Steven, Chao-Huan

Filed 13/12/2000

Issued 01/11/2001

Applicant

US20030185898 A1

Cartilage-Derived morphogenetic proteins

Inventor(s): Luyten, Frank, P.; Moos, Malcolm; Chang, Steven, Chao-Huan

Filed 1/5/2000

Published 2/10/2003

Applicant

US20010011131 A1

DNA molecules encoding cartilage-derived morphogenetic proteins

Inventor(s): Luyten, Frank, P.; Moos, Malcolm; Chang, Steven, Chao-Huan

Filed 5/12/2000

Issued 2/08/2001

Applicant

US20010039050 A1

Serum-free cell growth medium

Inventor(s): Luyten, Frank P.; Erlacher, Ludwig

Filed 9/11/2001

Issued 8/11/2001

Applicant The United States of America as represented by the Department of Health and Human Services

WO9859035 A2

Serum-free cell growth medium

Inventor(s): Luyten, Frank P.; Erlacher, Ludwig

Filed 22/06/1998

Published 30/12/1998

Applicant The United States of America as represented by the Department of Health and Human Services

US6617161 B2

Serum-free cell growth medium

Inventor(s): Luyten, Frank P.; Erlacher, Ludwig

Filed 09/05/2001

Issued 9/09/2003

Applicant The United States of America as represented by the Department of Health and Human Services

WO9816641 A1

Isolation and method of using tissue growth-inducing Frzb protein

Inventor(s): Luyten, Frank P.; Moos, Malcolm; Hoang, Bang; Wang, Shouwen

Filed 8/10/1997

Published 23/04/1998

Applicant The United States of America as represented by the Department of Health and Human Services

US20030009023 A1

Isolation and method of using tissue growth-inducing Frzb protein

Inventor(s): Luyten, Frank P.; Moos, Malcolm; Hoang, Bang; Wang, Shouwen

Filed 28/02/2002

Published 9/01/2003

Applicant The United States of America as represented by the Department of Health and Human Services

US20020147329 A1

Method of modulating tissue growth using Frzb protein

Inventor(s): Luyten, Frank P.; Moos, Malcolm; Hoang, Bang; Wang, Shouwen

Filed 19/12/2001

Published 10/10/2002

US20030139591 A1

Isolation and use of tissue growth-inducing Frzb protein

Inventor(s): Luyten, Frank P.; Moos, Malcolm; Hoang, Bang; Wang, Shouwen

Filed 07/12/2001

Published 24/07/2003

WO9614335 A1

CARTILAGE-DERIVED MORPHOGENETIC PROTEINS

Inventor(s): LUYTEN, Frank, P.; MOOS, Malcolm, Jr.; CHANG, Steven, Chao-Huan

Filed 19941107

Published 19960517

Applicant THE GOVERNMENT OF THE UNITED STATES OF AMERICA

TEACHING EXPERIENCE: DOCTORAL AND POSTDOCTORAL TRAINEES

- FAES advanced postdoctoral course in the Biochemistry of Connective Diseases, National Institutes of Health, Bethesda, MD 20892, USA, 1993.
- Seminars for summer students at the NIDR, National Institutes of Health, Bethesda, MD, USA, from 1995-1997.
- Summer Students (1-2 per year, 1992-1997).
- Promotor or Co-Promotor Doctoral Students:
 - Marco Helder, Ph.D., (1992-1993)
 - Bang Hoang, M.D., Howard Hughes Research Fellow, (1993-1995)
 - Steven Chang, M.D. (1993-1994)
 - Francesco Dell'Accio, M.D. (1997-2003)
 - Cosimo De Bari, M.D. (1997-2003)

Rik Lories, M.D. (1998-2003)
Jeroen Eyckmans (2001-present)
Marechal Marina (2003-2006)
Melina Daans (2003-present)
Giovanni Matricali (2003-present)
Nijs Stefaan (2003-present)
Bellon Ellen (2005-present)

- Postdoctoral Trainees:

Ping Chen, Ph. D. (1992-1995)
Sharon Tomaski, M.D. (1992-1993)
Keming Lin, M.D. (1994-1997)
Terrig Thomas, Ph.D. (1994-1997)
Ludwig Erlacher, M.D. (1995-1997)
Chee Keng Ng, Ph.D. (1996-1997)
Premyslav Tylzanowski, Ph.D. (1997-present)
Dirk De Valck, Ph.D. (1998-2004)
Rik Lories, M.D., Ph.D. (2003-present)
Astrid Bakker, Ph.D. (2004-present)

- Visiting Scholars:

Georges Zalzal, M.D., Associate Professor and Chair of the Department of Otolaryngology at the Children's National Medical Center, Washington D.C., USA (1992)
Slobodan Vukicevic, M.D., Professor, Department of Anatomy and Cell Biology, Zagreb University Medical School, Zagreb, Croatia (1992-1995)

EDITORIAL BOARD MEMBER:

- Annals of the Rheumatic Diseases
- Bone
- Journal of Dental Research

BIBLIOGRAPHY:

PEER REVIEWED INTERNATIONAL ARTICLES

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2. VERBRUGGEN G, VEYS EM, LUYTEN FP. Dedifferentiation of human Chondrocytes in monolayer culture. Clin Rheumatol 1984; 3: 97-8. IF:1.15
3. VERBRUGGEN G, LUYTEN F, VEYS EM. Repair function in organ-cultured human cartilage. Replacement of enzymatically removed proteoglycans during long-term organ culture. J Rheumatol 1985; 4: 665-74. IF:2.86
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CI:4
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CI:170
8. DE KEYZER F, VERBRUGGEN G, VEYS EM, LUYTEN F, SEGERS J, RABAEY M. Microgel immunoblotting of thymus and nuclear extracts by unidirectional diffusion. Anal Biochem 1989; 176: 350-2. IF:2.37
CI:5
9. LUYTEN FP, CUNNINGHAM NS, MA S, MUTHUKUMARUN R, HAMMONDS RG, NEVINS WB, WOOD WI, REDDI AH. Purification and partial amino acid sequence of osteogenin, a protein initiating bone cell differentiation. J Biol Chem 1989; 264: 13377-80. IF:6.36
CI:243
10. VUKICEVIC S, LUYTEN FP, REDDI AH. Stimulation of the expression of osteogenic and chondrogenic phenotypes in vitro by osteogenin. Proc Natl Acad Sci USA 1989; 86: 8793-7. IF:10.45
CI:181
11. REDDI AH, MUTHUKUMARAN N, MA S, CARRINGTON JL, LUYTEN FP, PARALKAR VM, CUNNINGHAM NS. Initiation of bone development by osteogenin and promotion by growth factors. Connect Tissue Res 1989; 20: 303-12. IF:1.15
CI:27

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CI:51
13. VUKICEVIC S, LUYTEN FP, KLEINMAN HK, REDDI AH. Differentiation of canalicular cell processes in bone cells by basement membrane matrix components: regulation by discrete domains of laminin. Cell 1990; 63: 437-45. IF:28.39
CI:113
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CI:31
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CI:82
16. ZALZAL GM, LUYTEN FP. An in vitro model for studying growth, and effects of trauma and external agents on the cricoid at the cellular level. Arch Otolaryngol 1992; 118: 407-11. IF:1.41
CI:4
17. HARRISON ET Jr, LUYTEN FP, REDDI AH. Transforming growth factor-beta: its effect on phenotype reexpression by dedifferentiated chondrocytes, in the presence and absence of osteogenin. In Vitro Cell and Dev Biology 1992; 28: 445-8. IF:0.39
CI:10
18. VUKICEVIC S, LUYTEN FP, KLEINMAN H, CUNNINGHAM N, ROBERTS A, REDDI AH. Growth factors in reconstituted basement membrane (Matrigel) modulate the network formation by immature osteoblastic cells. Exp Cell Res 1992; 202: 1-8. IF:4.01
CI:226
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CI:37
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CI:13
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CI:36
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CI:172
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CI:48
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CI:102
28. LUYTEN FP. Cartilage-derived Morphogenetic Proteins: Key Regulators in Chondrocyte differentiation ? Acta Orthop Scand Suppl., 1995, 66, 51-4. IF:1.02
CI:3
29. HOANG B, MOOS M, VUKICEVIC S, LUYTEN FP. Structure and Expression Pattern of Frzb, a Novel *frizzled* Related Protein, Suggest a Role in Skeletal Morphogenesis, J Biol Chem 1996; 271: 26131-7. IF:6.36
CI:79
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CI:14
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CI:88
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CI:163
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CI:9
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CI:251
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CI:94
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CI:2
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CI:38
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CI:51
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